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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
HUTSON, RICHARD G	
ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 11/17/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/037,633	SANLI ET AL.	
	Examin r	Art Unit	
	Richard G Hutson	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 September 2003*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-37,40-43 and 49-52* is/are pending in the application.

4a) Of the above claim(s) *19-37 and 49-52* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-18 and 40-43* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a) ☐ The translation of the foreign language provisional application has been received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Applicants amendment of claims 1-18 and 40-43, Paper of 9/8/2003, is acknowledged. Claims 1-37, 40-43 and 49-52 are still at issue and are present for examination.

Claims 19-37 and 49-52 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention, the requirement having been traversed in Paper No. 7.

Specification

The disclosure is objected to because of the following informalities:

Previously it was pointed out that Applicants abstract recites in a number of places "nucleic acid comprises" (line 5 and line 7) and "polypeptide, comprises" (line 9) and that this should be amended to "nucleic acid comprising" and "polypeptide, comprising". In response to the above suggestion, applicants have amended the abstract such as "nucleic acid [comprises] **includes**" (line 5 and line 7) and "polypeptide, [comprises] **includes**" (line 9). This amendment does not correct the problem, as the objection was not made because of the choice of "comprises" versus "includes", but rather "comprises" versus "comprising" (or "includes" versus "including").

Appropriate correction is required.

Claim Objections

Claims 2-9 are objected to because of the following informalities:

Claims 2-9 are objected to because applicants have amended each of these claims to recite the nucleic acid sequence of claim 1", without also amending claim 1 such that it is drawn to a "nucleic acid sequence" but rather applicants have left claim 1 such that it is drawn to a "nucleic acid comprising a sequence". While it is understood that a "nucleic acid comprising a sequence" is a "nucleic acid sequence", for the sake of consistency and in order to avoid any confusion it is suggested that applicants amend claim 1 such that it is drawn to a "nucleic acid sequence comprising a sequence..."

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 3, 6, 7, 8, 10-18 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In the previous office action, claim 10 (11-18 dependent on) was rejected as being indefinite in that it is confusing in that it is appears to be a duplicate of claim 1. Claim 10 is drawn to an isolated nucleic acid comprising a sequence having a GC content of from about 55% to 67% and encoding a polypeptide having the amino acid sequence of SEQ ID NO: 5. Claim 1 is drawn to drawn to an isolated nucleic acid comprising a degenerate variant of the nucleotide sequence of SEQ ID NO: 1 having a GC content of from about 55% to 67%. As SEQ ID NO: 1 encodes SEQ ID NO: 5, any

degenerate variant of SEQ ID NO: 1 must also encode SEQ ID NO: 5, and thus claim 1 is drawn to an isolated nucleic acid comprising a sequence having a GC content of from about 55% to 67% and encoding a polypeptide having the amino acid sequence of SEQ ID NO: 5. Thus claims 1 and 10 are duplicates as are each of the claims that depend from claims 1 and 10 duplicates of each other.

In response to this rejection applicants amended claim 10 and state that this amendment is such that claim 10 is not a duplicate of claim 1, which is clearly broader in scope than claim 10, however applicants amendment and argument are not persuasive and the rejection is maintained for the previous reasons of record.

Claims 40, 41, 42 and 43 have been withdrawn from this rejection based on applicants amendment.

Claim 18 remains indefinite in that the recitation "wherein the GC content is effective for producing an average codon bias in enteric bacteria of from greater than about 44% to about 66%" is unclear. It appears that applicants reference to "from greater than about 44% to about 66%" refers to the "average codon bias in enteric bacteria". How is codon bias related to a percentage??

Applicants have not responded to this previous rejection.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-18 and 40-43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Anderson et al. (Science, Vol 230, pages 144-149, 1985) and Mohsen et al. (Gene, Vol 160, pages 263-267, 1995).

The rejection was stated in the previous office action and repeated below.

Anderson et al. teach the isolation and cloning of the cDNA which encodes 2, 5-diketo-D-gluconic acid reductase A having the sequence of instantly disclosed SEQ ID NO: 1. It is noted that the GC content of the instantly disclosed SEQ ID NO: 1 is 68%.

Mohsen et al. teach the high-level expression of an altered cDNA encoding human isovaleryl-CoA dehydrogenase (IVD) in *Escherchia coli*. Specifically, Mohsen et al. teach that the cloned human IVD cDNA coding region includes a region with a high G + C content (73.5%). Mohsen et al. teach that bias codon usage in different organisms has been recognized as a possible mechanism for regulation of protein expression, and thus altered the codon usage of the region with high G + C content to mimic codon usage of highly expressed proteins in *E. coli* (See Figure 1A) as a means of increasing the expression of the cloned human IVD cDNA in *E. coli*. Mohsen et al. altered the IVD cDNA such that the G + C content in the corresponding are changed from 59.3% in the wildtype to 53.7% in the modified cDNA. The altered codons taught by Mohsen et al. included valine, alanine arginine and glycine.

One of ordinary skill in the art at the time of filing would have been motivated to express the cDNA taught by Anderson et al. in *E. coli* so that it could be easily

manipulated and expressed. One of ordinary skill in the art would have been further motivated to alter the codon usage of the cDNA taught by Anderson et al., SEQ ID NO: 1 such that the codon usage reflected those used routinely in *E. coli* so as to increase the production of the encoded 2, 5-diketo-D-gluconic acid reductase A protein. Such alterations in codon preference as taught by Mohsen et al. would result in a decrease in the G + C content to less then it is in the wildtype, which is 68%, thus the G + C content would be in the range of 55% to 67%. The many advantages of recombinant production of useful proteins in *E. coli* are well known within the art. These advantages include the ability to produce much larger quantities of the protein, being able to produce the protein in more easily handled organism, reducing the number of steps necessary for the purification of a protein and producing the protein in a purer form by using an organism that does not include naturally occurring contaminants of the protein. The reasonable expectation of success comes from the high level of skill in the art in the area of recombinant expression of proteins.

In response to this rejection applicants submit a declaration by co-inventor Michael Blaber and applicants arguments. Applicants submit that neither reference to Anderson et al. or to Mohsen et al. considers the problem addressed by the present invention. Applicants submit that Andersen et al. teach that poor expression of the wild-type DKGR gene in *E. coli* is due to the tertiary structure caused in the mRNA palindromic sequence and that the present invention has shown this to be incorrect, and that the poor expression is a result of the high G+C content of the gene. Thus

applicants submit that the Anderson et al. reference and its teachings would lead the person of skill in the art away from the present invention.

Applicants argument is not found persuasive for the following: First, Anderson et al. is merely relied upon for their teaching of the isolation and cloning of the cDNA which encodes 2, 5-diketo-D-gluconic acid reductase A having the sequence of instantly disclosed SEQ ID NO: 1. Andersen et al. further note that the sequence of the 2, 5-diketo-D-gluconic acid reductase A gene had a high G+C content, although they admittedly do not attribute this to any expression problems, rather they suggest the high G+C content may effect the sequencing of the gene and hence precautions were taken to ensure that the reading frame was correctly assigned. As previously noted the G+C content of the instantly disclosed SEQ ID NO: 1 is 68%. It is admitted that Anderson et al. noted that the 2, 5-diketo-D-gluconic acid reductase A gene did not contain a recognizable Shine-Dalgarno sequence in proper position and that the closest one that was identified was on the edge of a region of strong dyad symmetry that could mask translation of the gene. Anderson et al. however overcame this potential problem by deleting this region of the gene and replacing it with transcriptional and translational control sequences that work efficiently in E. coli (see top, right column of page 147). In spite of such measures, Anderson et al. note that "Surprisingly, expression of the 2, 5-DKG reductase was approximately 50 to 100 times higher in *E. herbicola* than in *E. coli* MM294 when the same expression plasmid (ptrp-35) was used." Thus there is some additional regulatory feature in addition to that pointed out by applicants that is

responsible a decreased level of expression in *E. coli*. While Anderson et al. may hypothesize what this may be, Anderson et al. admits that they do not know.

Applicants further assert that the Mohsen et al. reference also teaches away from the present invention or is in the alternative completely inapplicable in the present invention. Applicants assert that the IVD gene does not contain high G+C content throughout the gene, but concentrated in the 5' region. It is further noted by Dr. Blaber that in the 5' region of this gene is a mitochondrial targeting domain which is unnecessary for synthesis of the enzymatic portion of the IVD protein. Applicants assertions and Dr. Blabers statements regarding the teachings and any conclusions of Mohsen et al. are not considered persuasive or relevant to the present rejection.

As was previously stated, Mohsen et al. teach the high-level expression of an altered cDNA encoding human isovaleryl-CoA dehydrogenase (IVD) in *Escherchia coli*. Specifically, Mohsen et al. teach that the cloned human IVD cDNA coding region includes a region with a high G + C content (73.5%). Mohsen et al. teach that bias codon usage in different organisms has been recognized as a possible mechanism for regulation of protein expression, and thus altered the codon usage of the region with high G + C content to mimic codon usage of highly expressed proteins in *E. coli* (See Figure 1A) as a means of increasing the expression of the cloned human IVD cDNA in *E. coli*. Mohsen et al. altered the IVD cDNA such that the G + C content in the corresponding are changed from 59.3% in the wildtype to 53.7% in the modified cDNA. The altered codons taught by Mohsen et al. included valine, alanine arginine and glycine.

Applicants comments regarding conclusions regarding the "mitochondrial targeting domain" and its use in expressing proteins in *E. coli* are not considered relevant to the above rejection. In addition to the above and previous rejection, applicants attention is directed to the "Summary" of Mohsen et al. paper, which recites "To enhance expression, the nucleotide sequence of 22 codons within the 111-bp region at the end of the cDNA was altered to accommodate *E. coli* codon usage without altering the amino-acid coding sequence." This is the information and conclusion that the examiner is relying upon in making this rejection. Any reference to a mitochondrial targeting regions or problems associated with polymerase-based manipulations (e.g. PCR, or nucleic acid sequencing) are not considered relevant.

Conclusion

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Richard G Hutson whose telephone number is (703) 308-0066. The examiner can normally be reached on 7:30 am to 4:00 pm, M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on (703) 308-3804. The fax phone number for the organization where this application or proceeding is assigned is (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Richard G Hutson, Ph.D.
Primary Examiner
Art Unit 1652

rgh
11/6/2003